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Anti-inflammatory and Antinociceptive Activity of *Lawsonia inermis* Linn Alcoholic Extract in Rats.

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ABSTRACT: The present study investigates the anti-inflammatory activity of *Lawsonia inermis* Linn. ethanolic extract (LIAE) in carrageenan induced experimental model of acute inflammation. In an attempt to elucidate the mechanism behind the antiinflammatory activity of the plant extract, we evaluated its effects against inflammation produced by different mediators of carrageenan induced inflammation, *viz.* histamine, serotonin, bradykinin and PGE_2. As there are no studies reporting the central analgesic activity of ethanolic extracts of *Lawsonia inermis* in experimental models, we have also evaluated the central analgesic activity of LIAE using Eddy's hot plate method. LIAE administration produced a significant decrease in the paw edema produced by all the phologistic agents tested, thus demonstrating an effect on all phases of acute inflammation. In Eddy's hot plate method, LIAE significantly increased the latency of paw licking/jumping in the treated animals, suggesting a central analgesic action along with its anti-inflammatory effects. The present study thus demonstrates the potential for use of LIAE in the treatment of inflammatory disorders.

Keywords: Anti-inflammatory, carrageenan, histamine, serotonin, Lawsonia inermis, paw edema

INTRODUCTION

Inflammation is the body's protective reaction to any injury, with an aim to remove the inciting agent and repair the injured site. However, in a large number of human diseases, the underlying pathology is an uncontrolled inflammatory reaction that continues unchecked and induces permanent tissue destruction [1]. Currently, the mainstay for treatment of inflammatory conditions are NSAIDs and corticosteroids, which on chronic use are associated with a plethora of adverse effects including gastric ulceration, renal damage, cardiovascular complications and immunosuppression that affects the quality of life [2 - 5].

Therefore, there has been a constant search for newer alternatives that are similar in efficacy to the conventional agents, but without the burden of associated adverse effects. This has led to the screening of plant sources used in complimentary and alternative medicine (CAM) for potential anti-inflammatory principles.

Lawsonia inermis Linn. (Syn. *L. alba* Lam.) is a shrub belonging to Family Lythraceae, native to North Africa and Southwest Asia. It is commonly known as henna or mehandi and is widely cultivated in India, the Middle East and along the African coast of Mediterranean Sea for ornamental purposes and dye production [6]. In the traditional systems of medicine, *Lawsonia inermis* (LI) is used as an abortifacient, analgesic, anti-allergic, anti-microbial, anti-fungal, anti-inflammatory, anti-oxidant, aphrodisiac, astringent, blood purifier, brain tonic, diuretic, hepatoprotective and nootropic [6 - 8].

Despite widespread use in CAM for the treatment of inflammatory disorders, there is a dearth of studies that have scientifically evaluated the anti-inflammatory and analgesic properties of this drug. Ali *et al*, have evaluated the anti-inflammatory, antipyretic, and analgesic effects of chloroform, butanol, and water fractions of LI in experimental models [9] and have shown significant anti-inflammatory activity in the test plant. Similarly, Gupta *et al*, have demonstrated the antiinflammatory activity of phytochemical isolates from LI *viz*. luteolin, lawsone and 3-0-glucoside-β-sitosterol [10]. However, the polarities of water, chloroform/butanol are wide apart and therefore, the phytochemicals that are present in one fraction may not be present in the other. Ethanol has a polarity that lies between chloroform/butanol and water. Therefore, this fraction can be thought to contain phytochemicals that may overlap both chloroform/butanol and water fractions. Since no studies have been carried out to investigate the antiinflammatory activity of the ethanolic extract of LI, the present study was undertaken to scientifically evaluate the analgesic and anti-inflammatory activity of *Lawsonia inermis* alcoholic extract (LIAE) in experimental models. In our study, we used carrageenan as the principle model for evaluation of the acute antiinflammatory activity of the extract. Additionally, in order to understand the probable mechanism by which the test drug attenuates carrageenan induced paw edema, we also evaluated the activity of the extract in histamine, serotonin, bradykinin and PGE2 induced paw edema.

MATERIALS AND METHODS

Test Drug

Lawsonia inermis Linn. leaves were collected from Faridabad, Haryana and identified at the Department of

IJBLST

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Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard. A voucher specimen (SA-RM-001/2005) of the plant was submitted at the departmental herbarium for future reference. The leaves were air-dried at room temperature and reduced to a fine powder. The powder (1 kg) was packed in the form of a thimble and extracted using 3 L of ethanol for 6 hours in a Soxhlet apparatus. The crude extract was then evaporated to dryness under reduced pressure. A sticky mass was obtained and the yield was calculated to be 33.0% w/w.

Detection of Flavonoids in LIAE

The Shinoda/Pew test was performed for detecting the presence of flavonoids in the extract. This was followed by UV characterization to detect the different flavonoid isotype [11].

Animals

Male albino rats (Wistar strain) of 180–200g body weights, bred in the Central Animal House Facility, Jamia Hamdard, were used in the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and all experiments were carried out in accordance with 'Guidelines for care and use of animals in scientific research (Indian National Science Academy 1998, Revised 2000)'. The animals were kept under standard laboratory conditions at 25±1°C with a 12:12 hour light/dark cycle and were allowed access to food (Amrut Rat Feed, Pune, India) and water *ad libitum*. Animals were acclimatized for duration of 5 days before start of experimentation.

Table 1: Anti-inflammatory activity of LIAE in carrageenan induced paw edema in rats

Group	Increase in paw volume (ml) [% inhibition of paw swelling]					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I Control	0.76 ± 0.04	1.00 ±0.03	1.28 ± 0.02	1.20 ± 0.02	1.04 ± 0.02	0.86 ± 0.03
II Aspirin (100mg/kg)	0.18 ± 0.01 ** [76.31]	0.20 ± 0.02 ** [80.00]	0.28 ± 0.02 ** [78.12]	0.32 ± 0.03 ** [73.33]	0.28 ± 0.02 ** [73.07]	0.22 ± 0.01 ** [74.41]
III LIAE (0.75g/kg)	0.45 ± 0.01 ** [40.78]	0.60 ± 0.01 ** [40.00]	0.58 ± 0.02 ** [54.68]	0.49 ± 0.03 ** [59.16]	0.46 ± 0.03 ** [55.76]	0.38 ± 0.02 ** [55.81]

All data is mean ± SD. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ** P < 0.01

Table 2: Anti-inflammatory activity of LIAE in histamine induced paw edema in rats

Group	Increase in paw volume (ml) [% inhibition of paw swelling]					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I Control	0.52 ± 0.01	0.80 ± 0.02	0.94 ± 0.01	0.80 ± 0.02	0.59 ± 0.01	0.31 ± 0.02
II Chlor-pheniramine (8mg/kg)	0.12 ± 0.02 ** [76.92]	0.20 ± 0.02 ** [75.00]	0.21 ± 0.02 ** [77.65]	0.23 ± 0.02 ** [71.25]	0.13 ± 0.02 ** [77.96]	0.08 ± 0.01 ** [74.19]
III LIAE (0.75g/kg)	0.30 ± 0.01 ** [42.30]	0.45 ± 0.01 ** [43.75]	0.58 ± 0.02 ** [38.29]	0.61 ± 0.01 ** [23.75]	0.47 ± 0.02 ** [20.33]	0.30 ± 0.02 [3.22]

All data is mean ± SD. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ** P < 0.01

IJBLST

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Table 3: Anti-inflammatory activity of LIAE in serotonin induced paw edema in rats

Group	Increase in paw volume (ml) [% inhibition of paw swelling]					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I Control	0.66 ± 0.01	0.83 ± 0.02	0.90 ± 0.03	0.93 ± 0.02	0.71 ± 0.02	0.46 ± 0.02
II Cyproheptadine (10mg/kg)	0.06 ± 0.02 ** [90.90]	0.12 ± 0.01 ** [85.54]	0.16 ± 0.01** [82.22]	0.14 ± 0.02** [84.94]	0.09 ± 0.01** [87.32]	0.02 ± 0.01** [95.65]
III LIAE (0.75g/kg)	0.47 ± 0.01** [28.78]	0.58 ± 0.02 ** [30.12]	0.65 ± 0.01** [27.77]	0.68 ± 0.02** [26.88]	0.61 ± 0.01** [14.08]	0.36 ± 0.01** [21.73]

All data is mean ± SD. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ** P < 0.01

Table 4: Anti-inflammatory activity of LIAE in bradykinin induced paw edema in rats

Group	Increase in paw volume (ml) [% inhibition of paw swelling]					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I Control	0.45 ± 0.02	0.61 ± 0.01	0.70 ± 0.02	0.63 ± 0.01	0.46 ± 0.01	0.33 ± 0.01
II Aspirin (100mg/kg)	0.14 ± 0.01 ** [68.88]	0.16 ± 0.01 ** [73.77]	0.28 ± 0.02 ** [60.00]	0.16 ± 0.01 ** [74.60]	0.12 ± 0.01 ** [73.91]	0.11 ± 0.01 ** [66.66]
III LIAE (0.75g/kg)	0.23 ± 0.01 ** [48.88]	0.30 ± 0.02 ** [50.81]	0.41 ± 0.01 ** [41.40]	0.37 ± 0.01 ** [41.26]	0.28 ± 0.01 ** [39.13]	0.19 ± 0.02 ** [42.42]

All data is mean ± SD. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ** P < 0.01

Table 5: Anti-inflammatory activity of LIAE in prostaglandin E2 induced paw edema in rats

Group	Increase in paw volume (ml) [% inhibition of paw swelling]					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I Control	0.37 ± 0.01	0.50 ± 0.01	0.48 ± 0.02	0.35 ± 0.01	0.23 ± 0.01	0.08 ± 0.02
II Aspirin (100mg/kg)	0.18 ± 0.01 ** [51.35]	0.35 ± 0.01 ** [30.00]	0.37 ± 0.02 ** [22.91]	0.26 ± 0.01 ** [25.71]	0.19 ± 0.01 ** [17.39]	0.07 ± 0.01 [12.50]
III LIAE (0.75g/kg)	0.24 ± 0.01 ** [35.13]	0.34 ± 0.02 ** [32.00]	0.32 ± 0.01 ** [33.33]	0.33 ± 0.02 * [6.06]	0.20 ± 0.01 ** [13.04]	0.08 ± 0.01 [0.00]

All data is mean ± SD. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ** P < 0.01

IJBLST

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Table 6: Analgesic activity of LIAE in Eddy's hot plate model

Group	Increase in paw volume (ml)				
	30 min	60 min	90 min	120 min	150 min
I Control	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
II Aspirin (100mg/kg)	0.08 ± 0.03	0.06 ± 0.02	0.06 ± 0.03	0.08 ± 0.03	0.07 ± 0.04
III LIAE (0.75g/kg)	0.50 ± 0.02 **	0.87 ± 0.02 **	1.17 ± 0.04 **	1.03 ± 0.02 **	0.97 ± 0.05 **

All data is mean ± SD. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ** $P < 0.01$

Experimental Procedure

A pilot study was carried out using carrageenan challenge to determine a suitable anti-inflammatory dose of LIAE (data not shown). This dose (0.75g/kg body weight) was used for all subsequent analgesic and anti-inflammatory studies. All drugs were suspended in 1% gum acacia (which served as the vehicle) and were administered by gavage. All the phologistic agents (Sigma-Aldrich) were freshly prepared in sterile normal saline and were administered subcutaneously into the sub plantar surface of the hind paw using a sterile 26 gauge needle. Animals were divided into three groups (n=6). Group I received the vehicle (2ml/kg body weight) and served as the control, group II received the standard drug (depending on the phologistic agents used) and group III received LIAE (0.75g/kg body weight) for all analgesic and anti-inflammatory studies.

Anti-inflammatory activity of LIAE

Eighteen animals (three groups; n=6) were used for evaluation of the anti-inflammatory activity of LIAE against each phologistic agent. Grouping of animals was done as mentioned under experimental procedure. Group II received 100mg/kg body weight aspirin as the standard drug in carrageenan induced paw edema, bradykinin induced paw edema and prostaglandin E_2 (PGE_2) induced paw edema models. Chlorpheniramine (8mg/kg body weight) was used as the standard drug in histamine induced paw edema model and cyproheptadine (10mg/kg body weight) was used as the standard drug in serotonin induced paw edema model. Animals were administered drugs/vehicle once daily for duration of 3 days as pre-treatment. Exactly one hour after the third dose, volume of the right hind paw was measured by using a plethysmometer (Ugo Basile 7140). Thereafter, edema was induced by a sub plantar injection of 0.1 ml of either carrageenan (10×10^{-3} g/ml), histamine (1×10^{-3} g/ml), serotonin (1×10^{-3} g/ml), bradykinin (2×10^{-5} g/ml) or PGE_2 (1×10^{-6} g/ml) into the right hind paw, according to the method described by Winter et al. [12, 13]. The paw volume was then measured at one hour intervals upto 6 hours post phologistic agents administration.

Analgesic activity of LIAE

The analgesic activity of LIAE was evaluated using Eddy's Hot Plate method [14]. Forty eight hours before starting the experiment, animals were screened on the hot plate and eighteen animals showing reaction time (i.e., the time between placing the animal on the hot plate and its jumping or licking of the paws) between 4 – 6 seconds were chosen for the study. The reaction time of animals during screening was taken as the baseline reaction time. Grouping of animals was done as mentioned under experimental procedure and group II received the standard drug aspirin.

Animals were administered drugs/vehicle once daily for duration of 3 days as pre-treatment. Exactly thirty minutes after the dose on the third day, the animals were placed on a hot plate maintained at 55 ± 0.5°C and the reaction time was noted at 30, 60, 90, 120 and 150 minutes post drug/vehicle administration. To protect the animal from any thermal injury or tissue damage, a cut off time of 10 sec was strictly followed during all recordings.

Statistical Analysis

Difference between groups was compared by One-way ANOVA followed by Dunnett's Multiple Comparison. $P<0.05$ was considered to be significant.

RESULTS

Standardization of LIAE

Shinoda / Pew test for flavonoids was positive and the UV- characterization showed principal maxima at 272 nm indicating the presence of flavonols and absence of flavones. The total flavonol content in LIAE was found to be 8.06 µg/ml.

Anti-inflammatory activity of LIAE

In carrageenan induced paw edema model, an increase in the paw volume was seen in all the treated animals. Maximum swelling in the control animals was seen at 3 hours post carrageenan administration. Pre-treatment with LIAE (0.75g/kg) significantly inhibited carrageenan induced paw edema throughout the

observation period (Table 1). However, the inhibition of paw swelling by LIAE was less as compared to the standard drug aspirin.

In histamine induced paw edema model also, paw swelling was observed in all the animals throughout the observation period, with maximum swelling observed at three hours post histamine administration in the control animals (Table 2). Although inhibition of paw swelling was less as compared to the standard drug chlorpheniramine, pre-treatment with LIAE (0.75g/kg) significantly inhibited the increase in paw volume till five hour post histamine administration.

In the case of serotonin induced paw edema (Table 3), maximum paw edema was seen in the control groups at 4 hours post serotonin challenge. Both, LIAE and cyproheptadine produced significant inhibition of paw swelling throughout the observation period. As seen with other phologistic agents, the inhibition of paw edema by LIAE was less than that of the standard drug (cyproheptadine).

Similarly, in the bradykinin and PGE2 induced paw edema models also, an increase in paw volume was seen in all the animals throughout the observation period (Tables 4 and 5 respectively). Maximum swelling was observed in the control animals at 4 hours post phologistic agent administration. Even though LIAE significantly decreased the paw swelling as compared to control, the standard drug aspirin was superior to it except in the PGE2 induced edema model, where LIAE was more effective at 3 hours post PGE2 administration.

Analgesic activity of LIAE

In Eddy's hot plate test, there was a significant increase in the paw licking/jumping latency in the LIAE treated animals as compared to control throughout the duration of the study (Table 6). Although there was a marginal increase in the reaction time in the aspirin treated animals, it was not statistically significant as compared to the control. Maximum increase in reaction time was seen in the LIAE treated group at 90 minutes post drug administration.

DISCUSSION

Paw edema development in rodents is a very commonly used acute experimental model for the evaluation of anti-inflammatory agents. In this model, the anti-inflammatory activity of an agent is evaluated on the basis of its ability to inhibit edema formation in response to a phologistic agent. A large number of agents can be used for producing experimental paw edema, but the most commonly employed agent is carrageenan. Carrageenan induced paw edema has been shown to be a biphasic response, with the initial phase (0-2 hours) being primarily mediated by amino acid derived autacoids histamine and serotonin. The late phase (3-6 hours) is predominantly mediated by arachidonic acid derivatives (mainly prostaglandins), and kinins are involved in the transition between the two phases [15, 16, 17]. Therefore, the primary mechanism of an investigational agent is attributed to the inhibition of the autacoids that is predominantly acting during the phase when maximum anti-inflammatory activity is seen. However, the action of the individual autacoids does not terminate with each phase and continue throughout the duration of inflammation.

In the present study significant anti-inflammatory activity was seen with LIAE throughout the observation period, which suggests that LIAE has an inhibitory activity against all mediators of carrageenan induced inflammation. To further confirm the results, we evaluated the anti-inflammatory activity of LIAE individually against the different mediators. As demonstrated by the results, LIAE was effective in reducing the paw edema produced by all the phologistic agents tested, *viz*. histamine, serotonin, bradykinin and PGE2. This suggests that LIAE has an inhibitory activity against all mediators of acute inflammation.

Pain is one of the cardinal signs of inflammation, and therefore it is necessary to evaluate whether an anti-inflammatory agent also modifies pain. This is important because inflammatory pain is present in most of the conditions for which the anti-inflammatory drugs are used. Control of pain can either be by a peripheral mechanism (inhibition of inflammation), or a central mechanism (modulation of pain pathways). Since inhibition of arachidonic acid derivatives at the site of inflammation is the common mechanism behind both anti-inflammatory action and analgesia (peripheral mechanism), we only evaluated the central action of LIAE by using Eddy's hot plate in our study. As expected, the standard anti-inflammatory drug aspirin was not effective in this model. On the other hand, LIAE showed significant analgesic action as demonstrated by an increase in the reaction time, suggesting a potential central action along with the anti-inflammatory effect.

At this moment, it is difficult to attribute the anti-inflammatory and analgesic activity of LIAE to any specific phytochemical present in it. However, one chemical class that we believe is important for the above mentioned actions is flavonoids. Flavonoids are the most important constituents of alcoholic extract of *Lawsonia inermis* [18]. They are a diverse group of phytoconstituents which are broadly classified into three major subtypes, *viz.* flavonols, flavones and flavonones. Flavonoids have been reported to have

potent anti-oxidant and anti-inflammatory properties [19]. In our study, phytochemical evaluation of LIAE showed the presence of flavonols and absence of flavones.

Based on the current findings we believe that the flavonols present in the LIAE may be responsible for its anti-inflammatory effects. However, further studies with its individual flavonol fractions are required to identify the active principles that are responsible for the anti-inflammatory and analgesic actions. In conclusion, *Lawsonia inermis* alcoholic extract has the potential to be developed as an adjuvant for the treatment of inflammatory disorders.

REFERENCES

[1] Larsen G.L and P.M. Henson (1983) Mediators of Inflammation. Ann Rev Immunol, 1: 335-359.

[2] Nickander R., F.G. McMahon, A.S. Ridolfo (1979) Nonsteroidal anti-inflammatory agents. Ann Rev Pharmacol Toxicol, 19: 469-490.

[3] Rainsford K.D and M.W. Whitehouse (1980) Anti-inflammatory/ anti-pyretic salicylic acid esters with low gastric ulcerogenic activity. Agents Actions, 10: 451-454.

[4] Gault M.H., T.C. Rudwal, W.D. Engles, J.B. Dossetor (1968) Syndrome associated with the abuse of analgesics. Ann Intern Med, 68: 906-925.

[5] Arger P.H., E.I. Bluth, T. Murray, M. Goldberg (1976) Analgesic abuse nephropathy. Urology, 7: 123-128.

[6] Nadkarni K.M. (1976) Nadkarni's: Indian Materia Medica. Bombay, Popular Prakashan Pvt Ltd: 730.

[7] Ali M and S.H. Ansari. (1998) Studies on some herbal drugs used as hair tonic. Hamdard Medicus, 41: 107–108.

[8] Gupta N and L. Levy (1973) Delayed manifestation of ultraviolet reaction in the guinea pig caused by anti-inflammatory drugs. Br J Pharmacol, 47: 240-248.

[9] Ali B.H., A.K. Bashir, M.O. Tanira (1995) Anti-inflammatory, antipyretic, and analgesic effects of Lawsonia inermis L. (henna) in rats**.** Pharmacology, 51: 356-363.

[10] Gupta A., A.Q. Saifi, N.T. Modi, S.S. Rao, N. Mishra (1986) Antiinflammatory activity of some active principles of *Lawsonia inermis* leaves. Indian J Pharmacol, 18: 113-114.

[11] Dey P.M and J.B. Harborne (1987) Methods in Plant Biochemistry. London, Academic Press.

[12] Winter C.A., E.A. Risley, G.W. Nuss (1962) Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med, 111: 544-547.

[13] Winter C.A. and L. Flataker (1965) Nociceptive thresholds as affected by parenteral administration of irritants and of various antinociceptive drugs. J Pharmacol Exp Ther, 148: 373-379.

[14] Eddy N.B. and B. Leimbach (1953) Analgesic and antipyretic activities of Dalbergia sissoo leaves. J Pharmacol Exp Ther, 107: 385-393.

[15] Di Rosa M and D.A. Willoughby (1971) Screens for anti-inflammatory drugs. J Pharm Pharmacol, 23: 297-298.

[16] Di Rosa M, L. Sorrentino, L. Parente (1972) Non-steroidal anti-inflammatory drugs and leucocyte emigration. J Pharm Pharmacol, 24: 575-577.

[17] Vinegar R., W. Schreiber, R. Hugo (1969) Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther, 166: 96-103.

[18] Mahmoud Z.H., N.A. Abdel-salam, S.M. Khafegy (1980) Constituents of henna leaves (*Lawsonia inermis*) growing in Egypt. Fitoterapia, 51: 153-155.

[19] Selmi C., C.A. Cocchi, M. Lanfredini, C.L. Keen, M.E. Gershwin (2008) Chocolate at heart: The anti-inflammatory impact of cocoa flavanols. Mol Nutri Food Res, 52: 1340-1348.